Abstracts

• Fats and Oils

A NOVEL AND RAPID METHOD FOR THE PREPARATION OF METHYL ESTERS FOR GAS CHROMATOGRAPHY: APPLICATION TO THE DETERMINATION OF THE FATTY ACIDS OF EDIBLE FATS AND OILS. D.K. McCreary, *et al., J. Chromatogr. Sci.* 16, 329 (1978). A rapid, mild, and convenient transesterification of triglycerides was obtained using 0.2 *N*-methanolic (*m*-trifluoromethylphenyl) trimethylammonium hydroxide. The method yielded results comparable to those obtained using methanolic sodium methoxide.

BAKED PRODUCTS FOR THE FAT-CONTROLLED, LOW-CHOLESTEROL DIET. L. Fulton and C. Davis *J. Am. Diet. Assoc.* 73, 261 (1978). To help persons reduce dietary cholesterol and saturated fat, low-cholesterol formulas for white cake, sugar cookies, pastry, biscuits, muffins, and white yeast bread were tested with ¾ or 1 c. highly polyunsaturated oil replacing each cup of hydrogenated vegetable shortening. Cake, biscuits, muffins, and bread made with either level of oil were comparable in quality to products made with shortening. Cookies were best when oil replaced shortening cup for cup, and pastry was best when ¾ c. oil replaced 1 c. shortening. The effect of method of mixing on product quality often was greater than the effect of oil level.

RAMAN SPECTROSCOPY OF THE MILK GLOBULE MEMBRANE AND TRIGLYCERIDES. G. Forrest *Chem. Phys. Lipids* 21, 237–52 (1978). The acyl chain mobilities of the lipids of bovine milk fat globules and the component triglycerides have been determined by Raman spectroscopy as a function of temperature from −100°C to 80°C. A broad transition is observed centered about 2–6°C. The C–H and C–C stretching bands in the spectra of liquid and crystalline triglycerides are used comparatively to show that the lipids of the milk globule membrane are 30–40% more ordered than the lipids of the intact milk fat globules at 20°C. Synthetic triglyceride melts, quenched rapidly, are used to illustrate the effect of the thermal history of a sample on lipid order as determined spectroscopically.

FATTY ACIDS, PART XVI: THE SYNTHESIS OF ALL ISOMERIC C_{18} FURAN-CONTAINING FATTY ACIDS. M.S.F.L.K. Jie and C.H. Lam *Chem. Phys. Lipids* 21, 275–87 (1978). The synthesis of all isomeric C_{18} furan-containing fatty acids from furan, furfural or methyl octadecadiynoate is described.

ISOMERIZATION AND SATURATION OF MONOUNSATURATED ACYL MOIETIES DURING CATALYTIC HYDROGENATION INFLUENCED BY THEIR POSITION IN TRIACYLGLYCEROLS. M.M. Paulose, K.D. Mukherjee and I. Richter *Chem. Phys. Lipids* 21, 187–94 (1978). 1,2,3-Tri(Z)-9-octadecenoylglycerol (triolein) and 1,2,3-tri-(Z)-13-docosenoylglycerol (trierucin) were partially hydrogenated using a palladium catalyst. The unsaturated acyl moieties at the 2-position of 1,2,3-triacylglycerols were reduced at a slower rate than those at the 1,3-positions. The extent of geometrical isomerization was distinctly higher and the double bonds were somewhat more scattered in the acyl moieties at the 2-position than those at 1- and 3-positions.

NUCLEAR MAGNETIC RESONANCE AND LIGHT SCATTERING STUDIES OF THE AGGREGATION OF DIPALMITOYL-PHOSPHATIDYLCHOLINE BENZENE SYSTEMS. G. Klose, G. Hempel and TH.v. Zglinicki *Chem. Phys. Lipids* 21, 261–74 (1978). Magnetic resonance, light scattering measurements and visual observations have been performed on lecithin-benzene systems as a function of temperature from 80 to −60°C and concentration from $n = n_{benzene}/n_{DPPC} = 3$–200. From these measurements the transition curves from micellar solution to the crystalline state were deduced. More than 5 benzene molecules per lecithin molecule are necessary to get a micellar solution. The systems studied show considerable temperature hysteresis and ageing effects. Small amounts of water of one to two water molecules per lecithin molecule drastically change the properties of the lecithin systems.

• Biochemistry and Nutrition

EXPLORING THE ACTION AND SPECIFICITY OF COBRA VENOM PHOSPHOLIPASE A_2 TOWARD HUMAN ERYTHROCYTES, GHOST MEMBRANES, AND LIPID MIXTURES. M. Adamich and E.A. Dennis, *J. Biol. Chem.* 253, 5121–5 (1978). We have investigated the action and substrate specificity of phospholipase A_2 (EC 3.1.1.4) purified from cobra venom *(Naja naja naja)* toward intact and Triton-solubilized human erythrocytes, toward ghost membranes, and toward extracted ghost lipids in mixed micelles with Triton X-100. These results demonstrate a dependence of phospholipase A_2 activity on the ghosting procedure and a dependence of substrate specificity on the presence of other lipids. The relevance of these findings to the interpretation of membrane lipid asymmetry studies utilizing phospholipases is considered in detail.

HIGH LEVELS OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY AND CHOLESTEROL SYNTHESIS IN THE OVARY OF THE PREGNANT RABBIT. P.T. Kovanen, J.L. Goldstein, and M.S. Brown, *J. Biol. Chem.* 253, 5126–32 (1978). High levels of the rate-controlling enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), developed in the rabbit ovary during pregnancy. Enzyme activity in homogenates of whole ovary rose from 0.5 nmol/min/mg of microsomal protein in the nonpregnant state to a peak level of 3 $nmol/min^{-1}/mg^{-1}$ on Day 5 of pregnancy. The highest specific activity of HMG CoA reductase (20 $nmol/min^{-1}/mg^{-1}$) was observed in the corpus luteum on Day 5 of pregnancy. The current data demonstrate that a portion of the cholesterol substrate for steroid hormone synthesis during pregnancy is synthesized within the ovary and that this is associated with a marked enhancement in HMG CoA reductase activity in the corpus luteum.

LIPID REQUIREMENTS FOR ACTIVATION OF CTP:PHOSPHOCHOLINE CYTIDYLTRANSFERASE FROM RAT LIVER. P.C. Choy and D.E. Vance, *J. Biol. Chem.* 253, 5163–7 (1978). Phosphocholine cytidylyltransferase from rat liver is activated by incubation of cytosol at 4°C for several days or by the addition to enzyme preparations of a lipid extract from rat liver. We have investigated the nature of these two activations of this enzyme. The enzyme activity in fresh cytosol from rat liver was activated 10-fold by lysophosphatidylethanolamine and, to a lesser extent, by phosphatidylinositol and phosphatidylserine. Some species of lysophosphatidylcholine inhibited the enzyme activity in fresh cytosol by 80%.

N-(2-NAPHTHYL)-23,24-DINOR-5-CHOLEN-22-AMIN-3β-OL, A FLUORESCENT CHOLESTEROL ANALOGUE. Y.J. Kao *et al., Biochemistry* 17, 2689–96 (1978). Naturally occurring structural analogues of cholesterol were tested as substrates for lecithin: cholesterol acyltransferase and cholesterol oxidase. Minor differences in reactivity were associated with the nature of the 17β substituent of the sterol nucleus. The solvent-sensitive fluorescence properties of *N*-(2-naphthyl)-23,24-dinor-5-cholen-22-amin-3β-ol reveal a previously unrecognized role of cholesterol, i.e., the regulation of the polarity of the hydrocarbon *region of membranes.*

ACCELERATED PHOSPHOLIPID DEGRADATION AND ASSOCIATED MEMBRANE DYSFUNCTION IN IRREVERSIBLE, ISCHEMIC LIVER CELL INJURY. K.R. Chien *et al., J. Biol. Chem.* 253, 4809–17 (1978). Interruption of the blood supply to rat liver produces a progressive loss of phospholipids from the ischemic cells. Whole homogenates and post-mitochondrial supernatants from livers ischemic for 3 h showed a 40% and 55% decrease in phospholipids, respectively. Phosphatidylcholine and phos-

phatidylethanolamine were predominantly affected without accumulation of either lysophosphatidylcholine or lysophosphatidylethanolamine. Accelerated phospholipid degradation and its resultant membrane dysfunction are proposed as the critical alteration that produces irreversible liver cell injury and ultimately cell death in ischemia.

EFFECTS OF PLANT AND ANIMAL LIPIDS RICH IN DOCOSENOIC ACIDS ON THE MYOCARDIUM OF CYNOMOLGUS MONKEYS. F.M. Loew *et al., Nutr. Metab.* 22, 201–17 (1978). Cynomolgus monkeys *(Macaca fascicularis)* were fed diets containing 25% rapeseed oil (RSO), partially-hydrogenated herring oil (PHHO) or a 3:1 mixture of lard/corn oil as control (CON) for 4 months. The RSO contained approximately 25% of the fatty acids as erucic acid (*cis*-docos-13-enoic, 22:1w9) while the PHHO contained a similar concentration of mainly cetoleic acid (*cis*-docos-11-enoic, 22:1w11). The CON contained no 22:1 acids. The monkeys developed the expected myocardial lipidosis, somewhat more pronounced in the RSO than the PHHO group, but small foci of mononuclear cell infiltration, while infrequent, occurred in all three groups. Significant intergroup differences in biochemical or hematologic measurements of serum constituents were an increase in serum cholesterol concentration in the RSO group and an increase in serum glutamicoxaloacetic transaminase activity in both RSO and PHHO groups at certain intervals. The shorter proportion of *M. fasicularis* life span represented by this experiment may account for the absence of clear intergroup differences such as are reported in rats used in similar studies.

ACYL CHAIN LENGTH DEPENDENCY OF DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE AND DIACYLGLYCEROL ETHANOLAMINEPHOSPHOTRANSFERASE. EFFECT OF DIFFERENT SATURATED FATTY ACIDS AT THE C-1 OR C-2 POSITION OF DIACYLGLYCEROL ON SOLUBILIZED RAT LIVER MICROSOMAL ENZYMES. K. Morimoto and H. Kanoh, *J. Biol. Chem.* 253, 5056–60 (1978). A series of chemically synthesized diacylglycerols were tested as substrates for CDP-choline:1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) and CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.1). The enzymes were solubilized from rat liver microsomes. These results indicated that the influence of saturated fatty acids on the enzyme activities differs depending on whether they are located at the C-1 or C-2 position of diacylglycerol.

STIMULATING EFFECT OF HYDROCORTISONE ON THE CATABOLISM OF ENDOGENOUS FATTY ACYL GROUPS BY FATTY ACID-SUPPLEMENTED MOUSE L FIBROBLASTS. P. Tsai and R.P. Geyer, *J. Biol. Chem.* 253, 5087–9 (1978). Hydrocortisone stimulated the catabolism of prelabeled fatty acyls in mouse L fibroblasts supplemented with exogenous fatty acid. Both oxidation to $^{14}CO_2$ and release as free fatty acid from prelabeled lipids increased up to 20-fold under the described experimental conditions. The stimulating effect of hydrocortisone was observed even at concentrations as low as 1 μg/ml. The lipase acts on the triglycerides to liberate free fatty acids that are then oxidized to $^{14}CO_2$ or lost into the medium.

EFFECT OF DIETARY WHEAT GLUTEN ON LIPID METABOLISM IN GROWING RATS. S. Mokady and P. Einav, *Nutr. Metab.* 22, 181–9 (1978). The effect of dietary wheat gluten on liver and spleen lipogenesis in rats was studied *in vitro* and *in vivo*. Weanling rats were fed for 2 or 3 weeks an experimental diet containing wheat gluten as the only protein source and compared to other rats fed a casein control diet. Rats fed gluten showed enhanced *in vitro* lipogenesis as measured by conversion of (1-^{14}C)-acetate into liver and spleen lipids. These results indicated that the gluten-fed rats had a significantly higher hepatic capacity than the control rats to synthesize all lipid classes. On the other hand, the *in vivo* study of hepatic lipogenesis showed smaller differences between the group fed gluten and that fed casein. This suggests that the accumulation of lipids in fatty livers of gluten-fed rats is mostly due to increased rate of biosynthesis and not a result of impairment in the lipids' transport system. In the spleens of the gluten-fed groups, the enhanced *in vitro* lipogenesis was also found *in vivo*, indicating that accumulation of lipids in fatty spleens may be a result of biosynthesis only, with no other effects that can take place *in vivo*.

SUBSTRATE SPECIFICITY OF ENZYMES CATALYZING THE BIOSYNTHESIS OF IONIC ALKOXYLIPIDS FROM ALCOHOLS. Z.L. Bandi and H.K. Mangold, *Nutr. Metab.* 22, 190–9 (1978). An equimolar mixture of homologous saturated alcohols (13:0, 15:0, 17:0, 19:0) and an equimolar mixture of vinylogous alcohols (19:0, 19:1, 19:2) were fed to two groups of rats. All of the odd-numbered alcohols were incorporated into the ionic alkoxylipids of the rats' small intestine. Pronounced quantitative differences in the distribution of the various odd-numbered alkyl and alk-1-enyl moieties of choline phosphoglycerides and ethanolamine phosphoglycerides gave evidence to differences in the specificity of the enzyme system catalyzing the biosynthesis of alkoxylipids from long-chain alcohols.

APPARENT MICROVISCOSITY OF INTACT AND POST-LIPOLYSIS ("REMNANT") VERY LOW DENSITY LIPOPROTEIN PARTICLES. Y. Barenholz, A. Gafni, and S. Eisenberg *Chem. Phys. Lipids* 21, 179–85 (1978). The apparent microviscosity of intact rat plasma very low density lipoprotein (VLDL) and post-lipolysis very low density lipoprotein was determined by fluorescence depolarization measurements and fluorescence decay measurements using 1,6-diphenylhexatriene. It is concluded that the compositional changes occurring during lipolysis affect the physical properties of the lipoprotein, as measured here by the fluidity (microviscosity) of the particles.

ASSOCIATION OF THE MAJOR COAT PROTEIN OF FD BACTERIOPHAGE WITH PHOSPHOLIPID VESICLES. B.K. Chamberlain, *et al. Biochim. Biophys. Acta* 510, 18–37 (1978). The association of the major coat protein of fd bacteriophage with a phos-

pholipid bilayer was investigated by analyzing the protein's susceptibility to proteolysis and its circular dichroism spectrum when incorporated into singlewalled phospholipid vesicles. In the limits tested, this association appeared to be independent of the mass ratio of protein to lipid and of vesicle size, phospholipid composition, and method of preparation. The isolated core, which assumed a predominantly β-type conformation in detergent solution, maintained a β conformation when associated with a vesicle phospholipid bilayer.

CROSS-LINKING OF MEMBRANE PHOSPHOLIPID AND PROTEIN USING PUTATIVE MONOFUNCTIONAL IMIDOESTERS. R.C. Crain and G.V. Marinetti *Chem. Phys. Lipids* 21, 195–204 (1978). The reaction of methyl acetimidate or isethionyl acetimidate with mitoplasts at pH 8.5 yields two derivatives of phosphatidylethanolamine. These derivatives are shown to be the monoamidine derivative and the bis-derivative of phosphatidylethanolamine. The bis-derivative represents one phosphatidylethanolamine cross-linked to another phosphatidylethanolamine. Similar derivatives are formed by the reaction of dipalmitoyl phosphatidylethanolamine with these imidoesters in organic solution with the exception that much more monoderivative is produced. Putative monofunctional imidoesters cause considerable cross-linking of both phospholipids and proteins in cell membranes. Cross-linking can be minimized at pH 9.0.

POLYMYXIN BINDING TO CHARGED LIPID MEMBRANES AN EXAMPLE OF COOPERATIVE LIPID-PROTEIN INTERACTION. W. Hartmann, H.J. Galla and E. Sackmann *Biochim. Biophys. Acta* 510, 124–39 (1978). The binding of polymyxin-B to lipid bilayer vesicles of synthetic phosphatidic acid was studied using fluorescence, ESR spectroscopy and electron microscopy. 1,6-Diphenylhexatriene (which exhibits polarized fluorescence) and pyrene decanoic acid (which forms excimers) were used as fluorescence probes to study the lipid phase transition. A model is proposed which explains the association of polymyxin within the membrane plane in terms of elastic forces caused by the elastic distortion of the (liquid crystalline) lipid layer by this highly asymmetric peptide.

MEMBRANE LIPID MODIFICATIONS: BIOSYNTHESIS AND IDENTIFICATION OF PHOSPHATIDYL-*N*-METHYL-*N*-ISOPROPYLETHANOLAMINE IN RAT LIVER MICROSOMES. C. Moore *et al., Chem. Phys. Lipids* 21, 175–8 (1978). In previous studies on the modification of polar head groups of membrane phospholipids with the unnatural base analog, *N*-isopropylethanolamine, we reported an unidentified phospholipid in addition to phosphatidyl-*N*-isopropylethanolamine in the various membrane fractions of rat liver. The structure of this phospholipid has now been identified as phosphatidyl-*N*-methyl-*N*-isopropylethanolamine by nuclear magnetic resonance spectroscopy, and by chromatographic and enzymic analysis. In addition, we found that when rats were injected intraperitoneally with the *N*-methyl-*N*-isopropylethanolamine, 19% of the liver microsomal phospholipid was phosphatidyl-*N*-methyl-*N*-isopropylethanolamine.

STUDIES ON THE SURFACE STRUCTURE OF VERY LOW DENSITY LIPOPROTEINS. F.C. Reman, W. Nieuwenhuizen and T.M. Boonders *Chem. Phys. Lipids* 21, 223–35 (1978). Very low density lipoproteins (VLDL) were incubated with 5 different pure phospholipases. From the results the following conclusions were drawn. (1) The phospholipids are localized in a monomolecular layer on the outside of the VLDL particles. This supports the lipid core model proposed by several groups. (2) A minor fraction of the phospholipids (ranging from 3 to 10%) cannot be degraded by the enzymes and probably have a strong interaction with apoproteins. (3) The average surface pressure of VLDL is probably low, and comparable with a lateral surface pressure of 15 dynes/cm at the air-water interface, as concluded from experiments with two phospholipases A_2. Calculations of the thickness of the surface coat and protein coverage on the basis of this value agree very well with values reported in literature.

PROPERTIES OF SONICATED VESICLES OF THREE SYNTHETIC PHOSPHOLIPIDS. M.A. Roseman *et al., Chem. Phys. Lipids* 21, 205–22 (1978). The following synthetic phospholipids were prepared, and the structures that were formed by ultrasonic irradiation in aqueous solution were studied: 1,2-di(10-bromo

stearoyl)-3-*sn*-phosphatidylcholine (DBrPC), 1,2-di(10-methyl stearoyl)-3-*sn*-phosphatidylcholine (DMePC), and 1-palmitoyl-2-oleyl-3-*sn*-phosphatidylcholine (POPC). Uniform populations of small, unilamellar vesicles were obtained in all cases by gel filtration on Sepharose 4B. The partial specific volume of DMePC is larger than that of egg PC, whereas the partial specific volume of DBrPC is considerably lower; these lipids should therefore be useful in studies requiring the separation of vesicle populations. POPC, being virtually identical in size, shape and bilayer fluidity to egg PC, should be an excellent model of a 'natural' lecithin with a defined fatty acid composition.

THE INFLUENCE OF THREE SOURCES OF DIETARY FATS AND CHOLESTEROL ON LIPID COMPOSITION OF SWINE SERUM LIPIDS AND AORTA TISSUE. F.A. Kummerow *et al., Artery* 4, 360–84 (1978). The influence of three types of culinary fat, (I) two animal fats, (II) two hydrogenated vegetable *trans* fats and (III) two *trans*-free vegetable fats, on serum lipids and atherosclerosis was tested at a 37% calorie level from fat in a basal diet of 1,745 pounds of ground yellow corn, 200 pounds of defatted soybean meal and 55 pounds of a vitamin-mineral premix/ton. The impact of cholesterol was tested at a level of from 160 to 900 mg/day as crystalline cholesterol, egg yolk powder, whole egg powder, butterfat or beef tallow in a diet which either contained 8 or 37% calories from fat. These ten diet variations were fed for 8 months to 6 month old swine, the heparinized blood and aortas collected and the plasma and erythrocytes subjected to lipid analysis. The abdominal aorta of 7 out of 12 swine fed hydrogenated vegetable *trans* fat had raised lesions (58%), whereas raised lesions were observed in zero to three swine in each group or a total of 13 out of 92 of the abdominal aortas from the swine fed the other diets (14%).

THE INHIBITION OF α-KETOGLUTARATE OXIDATION BY FATTY ACIDS IN RAT LIVER MITOCHONDRIA. E. Lenartowicz and M.S. Olsen *J. Biol. Chem.* 253, 5990–6 (1978). The oxidation of α-ketoglutarate in rat liver mitochondria incubated in State 3 and in the uncoupled state in the absence and presence of oligomycin was inhibited by the simultaneous oxidation of fatty acids. Palmitylcarnitine inhibited α-ketoglutarate oxidation by up to 80% and octanoate by up to 30%. Various possibilities for the mechanism of this inhibition were experimentally evaluated.

ERYTHROCYTE LIPID COMPOSITION AND SODIUM TRANSPORT IN HUMAN LIVER DISEASE. J.S. Owen and N. McIntyre *Biochim. Biophys. Acta* 510, 168–76 (1978). In patients with liver disease there are usually increases in erythrocyte cholesterol and phosphatidylcholine concentrations. This increase in membrane lipid changes the shape of the erythrocyte and "spur" or "target" cells may be present. Sodium fluxes were measured in erythrocytes from 17 patients with a variety of liver diseases and from 17 normal subjects and the values related to the lipid content of the membrane. These results support the hypothesis that an altered lipid composition may affect the pereability of the erythrocyte membrane in patients with liver disease.

APOPROTEIN (E—A-II) COMPLEX OF HUMAN PLASMA LIPOPROTEINS. II. RECEPTOR BINDING ACTIVITY OF A HIGH DENSITY LIPOPROTEIN SUBFRACTION MODULATED BY THE APO(E—A-II) COMPLEX. T.L. Innerarity *et al., J. Biol. Chem.* 253, 6289–95 (1978). Normal human high density lipoproteins (HDL) of the d = 1.063 to 1.125 ultracentrifugal fraction can be separated by Geon-Pevikon block electrophoresis into two subclasses, HDL-I and HDL-II. HDL-I, characterized by the presence of the E apoprotein and the apo(E—A-II) complex along with the A-I and A-II apoproteins, accounted for most, if not all, of the high affinity binding of the human HDL (d = 1.063 to 1.21) to the low density lipoprotein (LDL) receptors on normal human fibroblasts. These studies show that the HDL-I subclass accounts for the binding activity of human HDL and that the activity of this subclass can be enhanced by conversion of the inactive apo(E—A-II) complex of HDL-I to the biologically active E apoprotein by reduction of the mixed disulfide. Whether or not this interconversion occurs *in vivo* and operates as a modulator of HDL binding to the LDL receptors remains to be determined.

STUDIES ON SERUM PRE-α-LIPOPROTEIN. A LIPOPROTEIN FAMILY WITH ONLY ONE APOLIPOPROTEIN PRESENT. L.E. Wille *Artery* 4, 330–47 (1978). Studies on pre-α-lipoprotein of human serum is presented, with special emphasis on the subfraction AAL(Fraction II_1). We report on electrophoretic studies, immunology, phospholipid and neutral lipid composition, amino acid composition, molecular weight studies, staining characteristics, results of polyanion precipitation, influence of urea, interaction with lysolecithin and the conditions in patients with lecithin:cholesterol acyl-transferase deficiency.

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